Studies on the searching behavior modified by olfactory conditioning in the German cockroach, *Blattella germanica* (L.)

I. Introduction

Learning is the process by which animals acquire knowledge on their environment. They alter their behavior through learning to better adapt to their surroundings. For example, through olfactory experiences, animals modify their behavior to improve efficiency in searching for resources. Olfactory learning in insects has proven to be an ideal model to study many aspects of learning and memory. However, most previous studies have focused only on the learning ability of insect species. Consequently, although changes in behavior have been used to assess learning in these studies, the impact of olfactory learning on behavior has been less well documented. To evaluate olfactory responses after learning quantitatively, the behavior itself should be analyzed. In the present study, I used the German cockroach, *Blattella germanica* (L.), a major urban pest, to investigate how olfactory learning modifies searching behavior in this model insect species.

II. Olfactory conditioning with single chemicals in the German cockroach, *Blattella germanica* (Dictyoptera: Blattellidae)

Studies of olfactory learning require manageable experimental conditions. A classical conditioning procedure that associates an odor with a taste allows the experimenter to manually control the sensory experience of the test animal. In particular, this approach allows the researcher to regulate the parameters of conditioned stimulus and unconditioned stimulus presentation, to modulate the responsiveness of the animal to the learned conditioned stimulus.

Initially, in this study, the classical differential olfactory conditioning procedure was confirmed in the German cockroach. In the appetitive conditioning trial, menthol odor was associated with sucrose solution. This was then followed by an aversive conditioning trial in which vanillin odor was associated with saline solution. However, while this approach, consisting of a differential conditioning trial followed by a preference test, provides adequate data for cognitive studies, it does not allow the researcher to assess the absolute potency of the odor in modifying the behavior of the animal after learning. Consequently, a standardized odor-training method, elementary conditioning, was established by modifying the differential conditioning to evaluate the attractiveness demonstrated by a single odor compound after olfactory conditioning. During training, (-)-menthol or vanillin odor was independently associated with sucrose (reward) or sodium chloride solution (punishment). The strength of the association with the odor was evaluated by determining the change in visit frequency to the odor source after olfactory conditioning. The frequency increased after (-)-menthol was presented with the reward, while it did not change when vanillin was presented with the reward. For both odors, the frequency significantly decreased after training with the punishment. These results indicate that cockroaches learn a single compound odor presented as a conditioned stimulus, although the association of the odor with a reward or punishment is dependent on the specific chemical. This olfactory conditioning method can be applied to the analysis of cockroach behavior elicited by a learned single odor.

III. Olfactory conditioning alters searching behavior in the German cockroach, *Blattella germanica* (Dictyoptera: Blattellidae)

Olfactory behavior in the German cockroach has been studied using not only semiochemicals, such as sex and aggregation pheromones, but also using general odors. However, whether the German cockroach searches for food in the same manner it searches for a mate or other conspecific is still unclear. To address this, the searching behavior of the cockroach evoked by a learned general odor was compared with that evoked by the aggregation pheromone in a circular arena under dim light illumination. Test animals choosing between odorous and clean-air zones were traced individually with a video tracking system. Cockroaches were localized to the semicircles permeated with menthol odor after reward-associated training. Similarly, cockroaches were localized to the semicircles permeated with the aggregation pheromone. However, right-about turns after crossing the odor boundary were observed only with the pheromone odor. When the odors were provided from the center of the test arena, cockroaches in the central zone increased kinetic responses, such as stay duration, track length and visit frequency, to both reward-associated menthol and the aggregation pheromone. These results indicate that while olfactory behaviors elicited by reward-associated menthol odor are similar to those elicited by the aggregation pheromone, they are not identical.

IV. Olfactory conditioning alters chemo-orientation in the German cockroach, *Blattella germanica* (Dictyoptera: Blattellidae)

Insects of many species orient to an odor source using an '*anemotactic*' wind-orienting system. To examine the effect of olfactory conditioning on orientation behavior in the German cockroach, walking behavior was recorded with a locomotion compensator, and the behavioral response of the insects to a periodic odor-loading airflow after classical olfactory conditioning was compared with that of untrained cockroaches. In clean air current, 86% of test animals walked downwind. Upon addition of menthol odor, 100% of the untrained cockroaches showed negative anemotaxis by walking away from the odor source. After six appetitive conditioning trials in which menthol odor was presented with sucrose solution, 26% of cockroaches walked upwind toward the odor source in the plume carrying menthol odor. This positive anemotaxis was not observed after the presentation of menthol odor (conditioned stimulus) or sucrose solution (unconditioned stimulus) alone.

V. Discussion

In this study, a classical olfactory conditioning method in insects was established. The behavioral test for learning performance was assessed using orientation behavior as a criterion. This experimental procedure was used to study the orientation mechanisms by which insects search an experienced odor. The findings in this study strongly suggest that the modification in searching behavior observed after olfactory conditioning is mainly caused by a switchover in anemotaxis after the rewarded learning. These results, as well as the methodology used in this study, should be useful in future studies of insect behavior.